PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 42968PCX329/29 KM	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. **PCT/NZ2004/000184**	International filing date *(day/month/year)* 13 August 2004	Priority date *(day/month/year)* 15 August 2003
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] A61K 31/407; A61P 9/10, 9/12		
Applicant AGRESEARCH LIMITED et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of **17** sheets, as follows:
      - [X] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - [X] Box No. I Basis of the report
   - [ ] Box No. II Priority
   - [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - [ ] Box No. IV Lack of unity of invention
   - [X] Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - [ ] Box No. VI Certain documents cited
   - [ ] Box No. VII Certain defects in the international application
   - [ ] Box No. VIII Certain observations on the international application

Date of submission of the demand 1 February 2005	Date of completion of the report 26 July 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **S. Chew** Telephone No. (02) 6283 2248

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NZ2004/000184

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1 (b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

☐ the international application as originally filed/furnished

☒ the description:

pages **1-33** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

☒ the claims:

pages as originally filed/furnished
pages* as amended (together with any statement) under Article 19
pages***34-50** received by this Authority on **1 February 2005** with the letter of **1 February 2005**
pages* received by this Authority on with the letter of

☒ the drawings:

pages **1/5 – 5/5** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages
☐ the claims, Nos.
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to the sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages
☐ the claims, Nos.
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to the sequence listing (*specify*):

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Statement

Novelty (N)	Claims **1-49**	**YES**
	Claims	**NO**
Inventive step (IS)	Claims **1-49**	**YES**
	Claims	**NO**
Industrial applicability (IA)	Claims **1-49**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

This report has considered the following documents cited in the International Search Report:

D1 WO 2003/105868

D2 Miles C. et al.

D3 Munday-Finch S. et al. J. Agric. Food Chem. 1995

D4 Munday-Finch S. et al. J. Agric. Food Chem. 1998

D5 Munday-Finch S. et al. J. Agric. Food Chem. 1997

D6 Derwent Abstract Accession No. 92-308267/38

D7 Munday-Finch S. et al. J. Agric. Food Chem. 1996

NOVELTY (N), INVENTIVE STEP (IS) : Claims 1-49

D1 discloses lolitrems A, B, C, E, F, H, N, lolitrem N-31-epimer, lolitriol, lolilline, lolitriol , lolicines A and B and their use as potassium channel blockers for the treatment of ocular hypertension or glaucoma (see pages 5, 7, 13 and claim 1).

D2 discloses the isolation and structures of lolitrems B and E including their biosynthetic route from lolitriol (see abstract and figure 1).

D3 discloses the isolation of lolitrem A, its structure and structures of lolitrems B, C and E (see abstract and figure 1).

D4 discloses the isolation of lolicines A and B, lolitriol and lolitrem N and has provided evidence for 31-epilolitrem N and 31-epilolitrem F (see abstract and figure 1).

D5 discloses lolilline, lolitrems A, B, E and lolitriol (see figures 1 and 3).

D6 discloses some lolitrem derivatives used for the preparation of haptens for the production of antibodies.

D7 discloses lolitrem F, lolitrem B, 31-epilolitrem B, 31-epilolitrem F and lolitriol (see abstract, figures 1 and 4).

Supplemental Box

case the space in any of the preceding boxes is not sufficient.

Continuation of Box No. V:

None of D1-D7 disclose or fairly suggest alone or in combination, a method of preventing repolarisation or hyperpolarisation of a cell wherein the cell contains a BK channel, comprising the administration to the cell of a composition containing a BK channel antagonist as defined in the claims, or a composition comprising a BK channel antagonist compound containing the moiety shown in structures (VII), (IX), (XII) and (XIII).

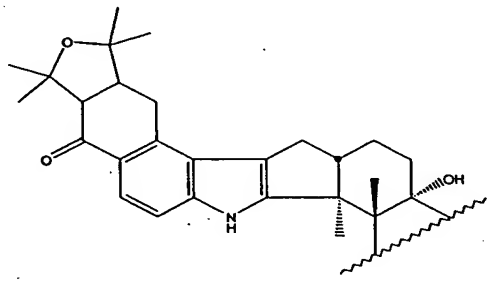
Therefore claims 1-49 are novel and have an inventive step.

INDUSTRIAL APPLICABILITY (IA): Claims 1-49

Claims 1-49 have industrial applicability.

WHAT WE CLAIM IS:

1. A method of preventing repolarisation or hyperpolarisation of a cell, wherein the cell contains a BK channel, including the administration to the cell of at least one pharmacologically effective amount of composition containing a BK channel antagonist containing the moiety shown in structure (I):



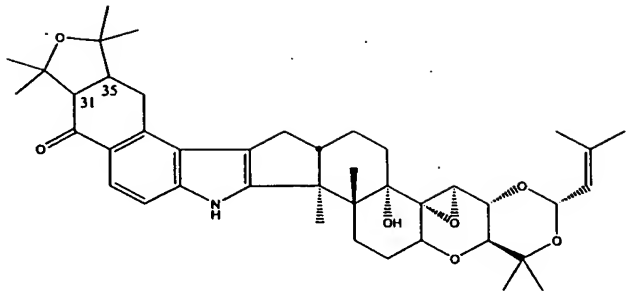
STRUCTURE (I)

or derivatives thereof.

2. The method as claimed in claim 1 wherein the derivatives of structure (I) are selected from the group consisting of: salts, analogues, isomers, and combinations thereof.

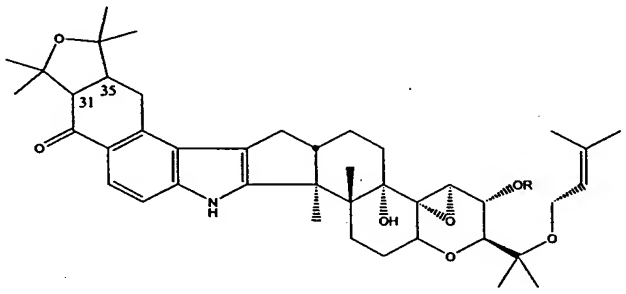
3. The method as claimed in claim 1 or claim 2 wherein the antagonist compound is selected from the group consisting of: lolitrem B, lolitrem A, lolitrem F, 31-*epi*lolitrem F, 31-*epi*lolitrem B, lolitrem E, lolitrem E acetate, lolitrem L, lolitrem G, lolitrem C, lolitrem M, lolitriol, lolitriol acetate, lolitrem N, lolitrem J, lolitrem H, lolitrem K, lolicine A and B, 30-desoxy lolitrem B-30α-ol, 30-desoxy-31-*epi*lolitrem B-30α-ol, 30-desoxylolitrem B-30-ene lolilline and combinations thereof.

4. The method as claimed in claim 1 or claim 2 wherein the antagonist compound is selected from the group consisting of:



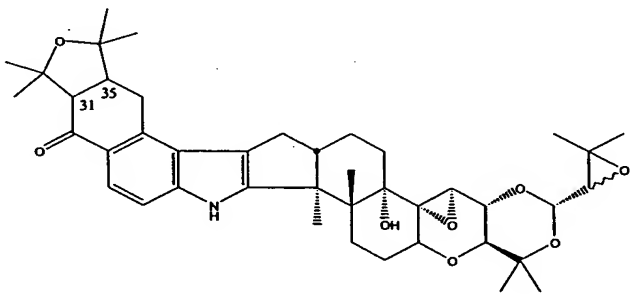
STRUCTURE (II)

which includes compounds selected from the group consisting of: lolitrem B = 31α, 35β stereochemistry; 31-*epi*lolitrem B = 31β, 35β stereochemistry; lolitrem F = 31α, 35α; 31-*epi*lolitrem F = 31β, 35α;



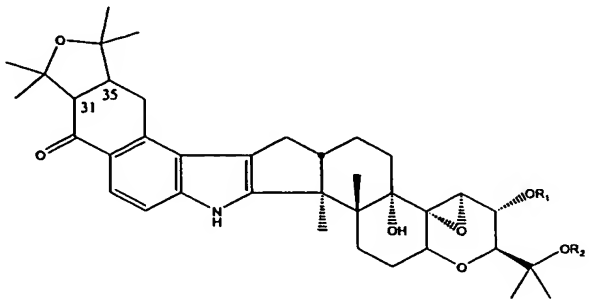
STRUCTURE (III)

which includes compounds selected from the group consisting of: lolitrem E = 31α, 35β stereochemistry where R = H or acetate; lolitrem L = 31α, 35α stereochemistry where R = H or acetate;



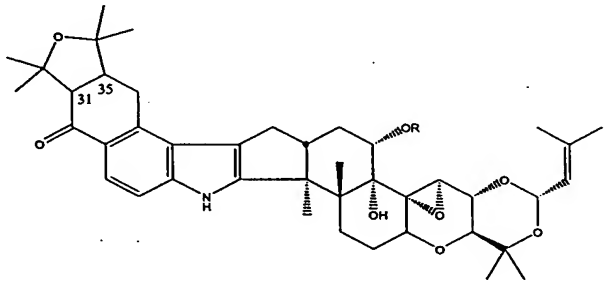
STRUCTURE (IV)

which includes compounds selected from the group consisting of: lolitrem A = 31α, 35β stereochemistry; lolitrem G = 31α, 35α stereochemistry;



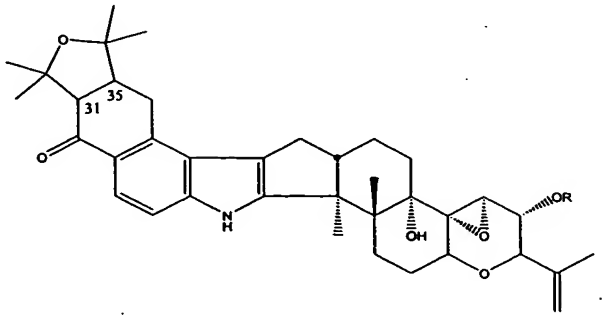
STRUCTURE (V)

which includes compounds selected from the group consisting of: lolitriol; = 31α, 35β stereochemistry where R_1 = H or acetate and R_2 = H; lolitrem N = 31α, 35α stereochemistry where R_1=H or acetate and R_2=H; Lolitrem J = 31α, 35β stereochemistry where R_1 = H or acetate and R_2 = acetate;



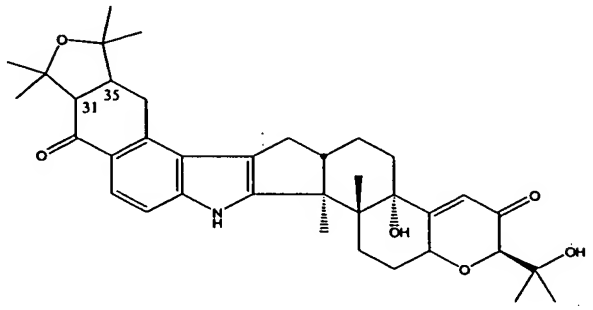
STRUCTURE (VI)

which includes lolitrem H = 31α, 35β stereochemistry where R = H or acetate;



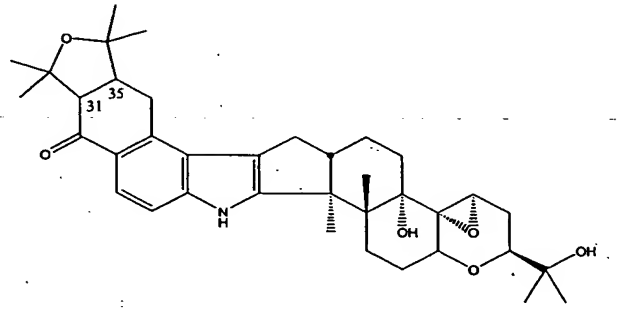
STRUCTURE (VII)

which includes lolitrem K = 31α, 35β stereochemistry, where R = H or acetate;



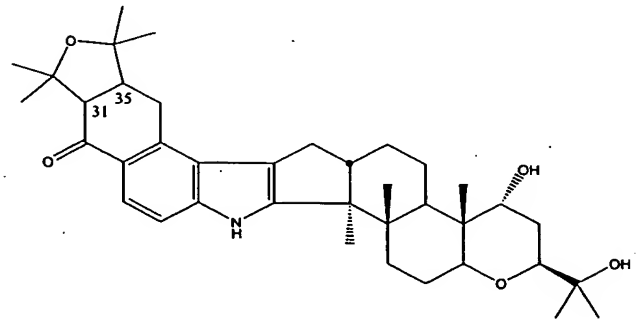
STRUCTURE (VIII)

which includes lolilline = 31α, 35β stereochemistry;



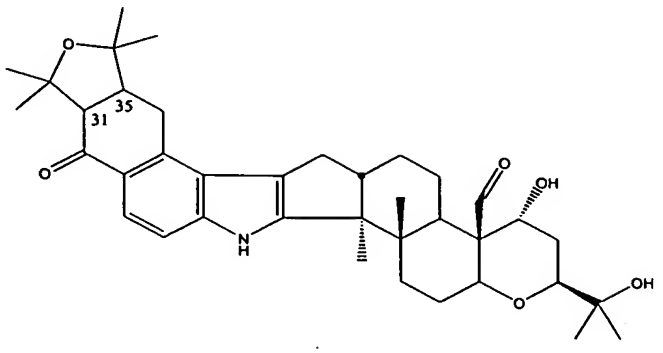
STRUCTURE (IX)

which includes lolitrem M = 31α, 35β stereochemistry;



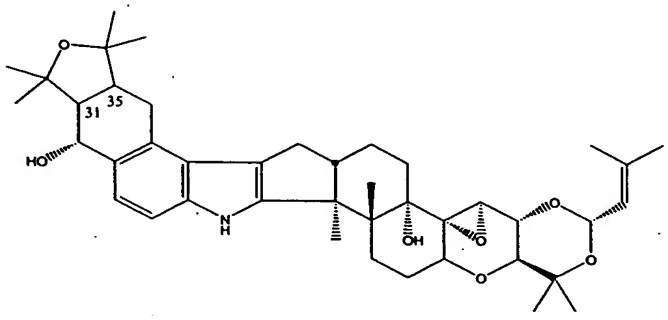
STRUCTURE (X)

which includes lolicine A = 31α, 35β stereochemistry;



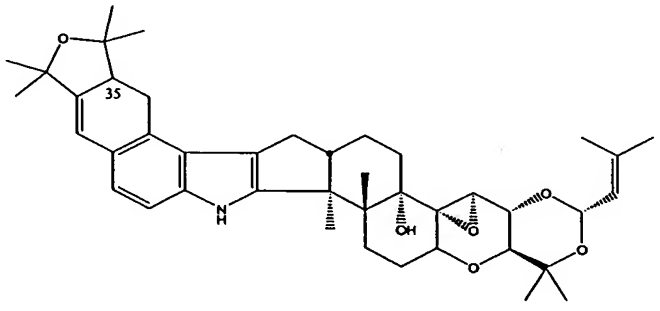
STRUCTURE (XI)

which includes lolicine B = 31α, 35β stereochemistry;



STRUCTURE (XII)

which includes compounds selected from the group consisting of: 30-desoxylolitrem B-30α-ol = 31α, 35β stereochemistry; 30-desoxy-31-*epi*lolitrem B-30α-ol = 31β, 35β stereochemistry;



STRUCTURE (XIII)

which includes 30-desoxylolitrem B-30-ene = 35β stereochemistry;

and combinations of the above compounds.

4. The method as claimed in any of the above claims wherein the composition further includes pharmaceutically and physiologically acceptable carriers.

5. The method as claimed in claim 4 wherein the pharmaceutically and physiologically acceptable carriers include components selected from the group including; fillers; excipients; modifiers; humectants; stabilisers; emulsifiers; diluents; and other formulation components such as a use of a lipid vehicle.

6. The method as claimed in any of the above claims wherein the composition is administered in a form selected from the group including: an injection; a tablet; a capsule; a suppository; an injection; a suspension; a drink or tonic; a syrup; a powder; an ingredient in solid or liquid foods; a nasal spray; a sublingual wafer; a transdermal patch; a transdermal injection; and combinations thereof.

7. The method as claimed in any of the above claims wherein the BK channel antagonist compound or compounds are extracted from endophyte-infected plants and seeds.

8. The method as claimed in any of claims 1 to 6 wherein the BK channel antagonist compound or compounds are extracted from fungal cultures.

9. The method as claimed in any of claims 1 to 6 wherein the BK channel antagonist compound or compounds are derived by chemical synthesis.

10. The method as claimed in any of claims 1 to 6 wherein the BK channel antagonist compound or compounds are extracted from heterologous expression systems including but not limited to bacteria, yeast, fungi, plants and animal cells.

11. The method as claimed in claim 7 wherein the perennial ryegrass seed is from

Lolium perenne.

12. The method as claimed in any of the above claims wherein the BK channel antagonist compound or compounds has activity against both alpha (α) subunit and alpha plus beta (β) accessory subunit (β_1 to β_4) channels.

13. The method as claimed in any of claims 1 to 4 wherein, for lolitrem B, the degree of antagonist inhibition is approximately 97% for a composition containing approximately 20nM lolitrem B.

14. The method as claimed in any of claims 1 to 4 wherein, for lolitrem B, the half maximal degree of antagonist inhibition (IC_{50}) is found for a composition containing approximately 3.7 ± 0.4 nM of lolitrem B.

15. The method as claimed in any of claims 1 to 4 wherein, for lolitriol, the degree of antagonist inhibition is approximately 100% for a composition containing approximately 1000 nM lolitriol.

16. The method as claimed in any of claims 1 to 4 wherein, for lolitriol, the half maximal degree of antagonist inhibition (IC_{50}) is found for a composition containing approximately 195 nM of lolitriol to inhibit α and β_1 BK channel activity

17. The method as claimed in any of claims 1 to 4 wherein, for lolitriol, the half maximal degree of antagonist inhibition (IC_{50}) is found for a composition containing approximately 536 ±16 nM of lolitriol to inhibit α and β_4 activity.

18. The method as claimed in any of claims 1 to 4 wherein, for 31-*epi*lolitrem B, the degree of antagonist inhibition is approximately 100% for a composition containing approximately 200nM 31-*epi*lolitrem B.

19. The method as claimed in any of claims 1 to 4 wherein, for 31-*epi*lolitrem B, the half maximal degree of antagonist inhibition (IC_{50}) is found for a composition

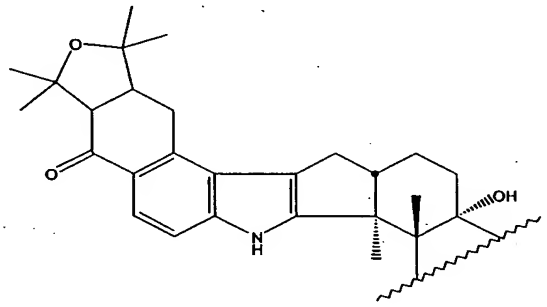
containing approximately 58 ±6 nM of 31-*epi*lolitrem B to inhibit α and β_1 activity.

20. The method as claimed in any of claims 1 to 4 wherein, for 31-*epi*lolitrem B, the half maximal degree of antagonist inhibition (IC_{50}) is found for a composition containing approximately 49 nM of 31-*epi*lolitrem B to inhibit α and β_4 activity.

21. The method as claimed in any of claims 1 to 4 wherein, for lolitrem E, the degree of antagonist inhibition is approximately 100% for a composition containing approximately 100 nM lolitrem E.

22. The method as claimed in any of claims 1 to 4 wherein the antagonist effect of the composition is not able to be reversed by wash out for concentrations of 10 nM or greater of lolitrem B compound.

23. Use of a composition for preventing repolarisation or hyperpolarisation of a cell that contains a BK channel wherein a pharmacologically effective amount of the composition is administered to the cell and wherein the composition contains at least one BK channel antagonist of the moiety shown in structure (I):



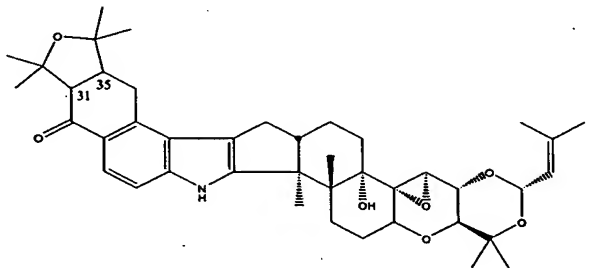
STRUCTURE (I)

or derivatives thereof.

24. The use as claimed in claim 23 wherein the derivatives of structure (I) are selected from the group consisting of: salts, analogues, isomers, and combinations thereof.

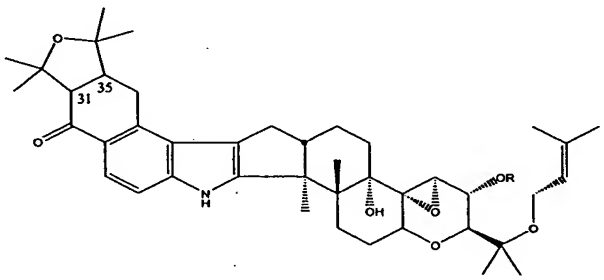
25. The use as claimed in claim 23 or claim 24 wherein the antagonist compound is selected from the group consisting of: lolitrem B, lolitrem A, lolitrem F, 31-*epi*lolitrem F, 31-*epi*lolitrem B, lolitrem E, lolitrem E acetate, lolitrem L, lolitrem G, lolitrem C, lolitrem M, lolitriol, lolitriol acetate, lolitrem N, lolitrem J, lolitrem H, lolitrem K, lolicine A and B, 30-desoxy lolitrem B-30α-ol, 30-desoxy-31-*epi*lolitrem B-30α-ol, 30-desoxylolitrem B-30-ene lolilline and combinations thereof.

26. The use as claimed in claim 23 or claim 24 wherein the antagonist compound is selected from the group consisting of:



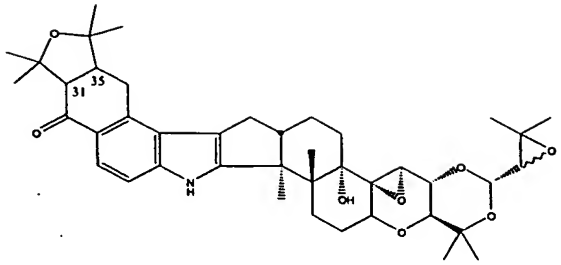
STRUCTURE (II)

which includes compounds selected from the group consisting of: lolitrem B = 31α, 35β stereochemistry; 31-*epi*lolitrem B = 31β, 35β stereochemistry; lolitrem F = 31α, 35α; 31-*epi*lolitrem F = 31β, 35α;



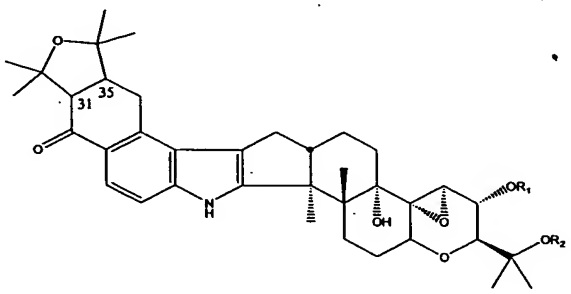
STRUCTURE (III)

which includes compounds selected from the group consisting of: lolitrem E = 31α, 35β stereochemistry where R = H or acetate; lolitrem L = 31α, 35α stereochemistry where R = H or acetate;



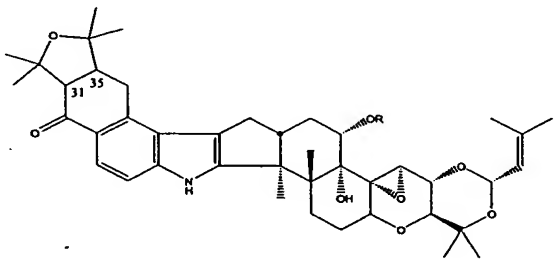
STRUCTURE (IV)

which includes compounds selected from the group consisting of: lolitrem A = 31α, 35β stereochemistry; lolitrem G = 31α, 35α stereochemistry;



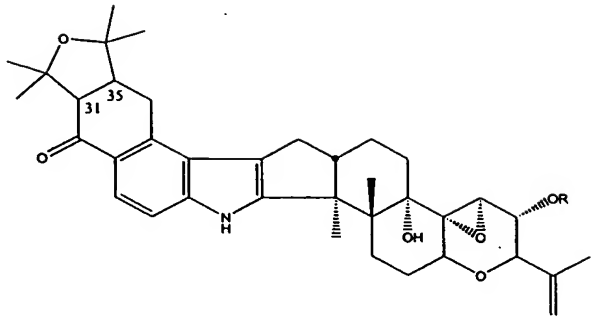
STRUCTURE (V)

which includes compounds selected from the group consisting of: lolitriol; = 31α, 35β stereochemistry where R_1 = H or acetate and R_2 = H; lolitrem N = 31α, 35α stereochemistry where R_1=H or acetate and R_2=H; Lolitrem J = 31α, 35β stereochemistry where R_1 = H or acetate and R_2 = acetate;



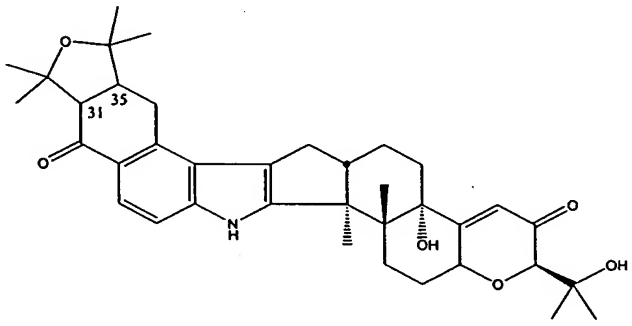
STRUCTURE (VI)

which includes lolitrem H = 31α, 35β stereochemistry where R = H or acetate;



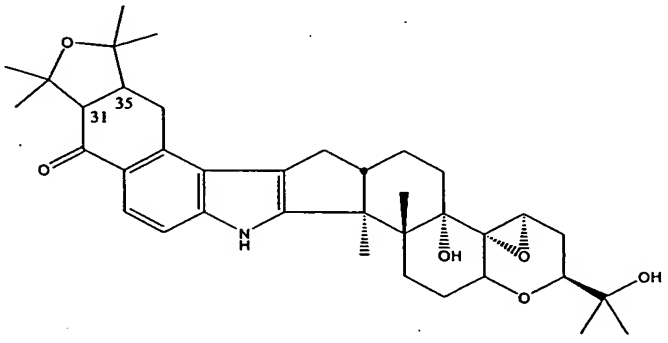
STRUCTURE (VII)

which includes lolitrem K = 31α, 35β stereochemistry, where R = H or acetate;



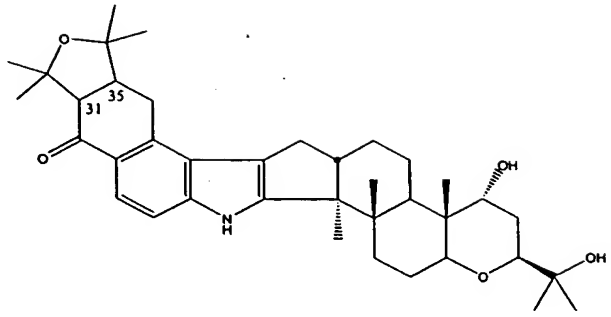
STRUCTURE (VIII)

which includes lolilline = 31α, 35β stereochemistry;



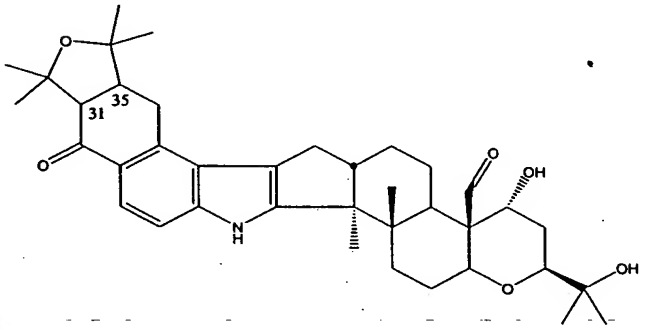
STRUCTURE (IX)

which includes lolitrem M = 31α, 35β stereochemistry;



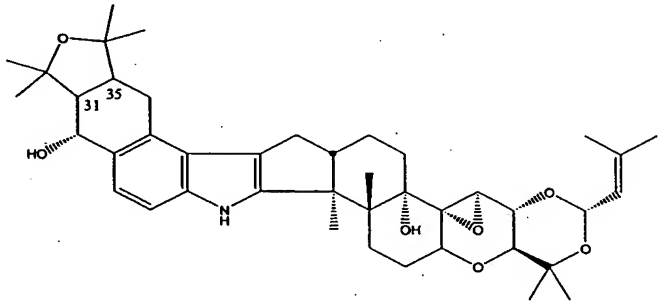
STRUCTURE (X)

which includes lolicine A = 31α, 35β stereochemistry;



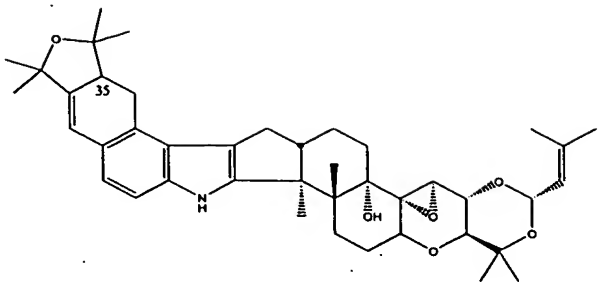
STRUCTURE (XI)

which includes lolicine B = 31α, 35β stereochemistry;



STRUCTURE (XII)

which includes compounds selected from the group consisting of: 30-desoxylolitrem B-30α-ol = 31α, 35β stereochemistry; 30-desoxy-31-*epi*lolitrem B-30α-ol = 31β, 35β stereochemistry;



STRUCTURE (XIII)

which includes 30-desoxylolitrem B-30-ene = 35β stereochemistry;

and combinations of the above compounds.

27. The use as claimed in any of the above claims wherein the composition further includes pharmaceutically and physiologically acceptable carriers.

28. The use as claimed in claim 27 wherein the pharmaceutically and physiologically acceptable carriers include components selected from the group including; fillers; excipients; modifiers; humectants; stabilisers; emulsifiers; diluents; and other formulation components such as a use of a lipid vehicle.

29. The use as claimed in any of claims 23 to 28 wherein the composition is administered in a form selected from the group including: an injection; a tablet; a capsule; a suppository; an injection; a suspension; a drink or tonic; a syrup; a powder; an ingredient in solid or liquid foods; a nasal spray; a sublingual wafer; a transdermal patch; a transdermal injection; and combinations thereof.

30. The use as claimed in any of claims 23 to 29 wherein the BK channel antagonist compound or compounds are extracted from endophyte-infected plants and seeds.

31. The use as claimed in any of claims 23 to 29 wherein the BK channel antagonist compound or compounds are extracted from fungal cultures.

32. The use as claimed in any of claims 23 to 29 wherein the BK channel antagonist compound or compounds are derived by chemical synthesis.

33. The use as claimed in any of claims 23 to 29 wherein the BK channel antagonist compound or compounds are extracted from heterologous expression systems including but not limited to bacteria, yeast, fungi, plants and animal cells.

34. The use as claimed in claim 30 wherein the perennial ryegrass seed is from *Lolium perenne*.

35. The use as claimed in any of claims 23 to 34 wherein the BK channel antagonist compound or compounds has activity against both alpha (α) subunit and alpha plus beta (β) accessory subunit (β_1 to β_4) channels.

36. The use as claimed in any of claims 23 to 26 wherein, for lolitrem B, the degree of antagonist inhibition is approximately 97% for a composition containing approximately 20nM lolitrem B.

37. The use as claimed in any of claims 23 to 26 wherein, for lolitrem B, the half maximal degree of antagonist inhibition (IC_{50}) is found for a composition containing approximately 3.7 ± 0.4 nM of lolitrem B.

38. The use as claimed in any of claims 23 to 26 wherein, for lolitriol, the degree of antagonist inhibition is approximately 100% for a composition containing approximately 1000 nM lolitriol.

39. The use as claimed in any of claims 23 to 26 wherein, for lolitriol, the half maximal degree of antagonist inhibition (IC_{50}) is found for a composition containing approximately 195 nM of lolitriol to inhibit α and β_1 BK channel activity

40. The use as claimed in any of claims 23 to 26 wherein, for lolitriol, the half maximal degree of antagonist inhibition (IC_{50}) is found for a composition containing

approximately 536 ±16 nM of lolitriol to inhibit α and β_4 activity.

41. The use as claimed in any of claims 23 to 26 wherein, for 31-*epi*lolitrem B, the degree of antagonist inhibition is approximately 100% for a composition containing approximately 200nM 31-*epi*lolitrem B.

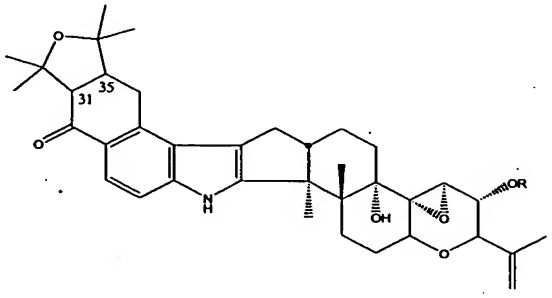
42. The use as claimed in any of claims 23 to 26 wherein, for 31-*epi*lolitrem B, the half maximal degree of antagonist inhibition (IC_{50}) is found for a composition containing approximately 58 ±6 nM of 31-*epi*lolitrem B to inhibit α and β_1 activity.

43. The use as claimed in any of claims 23 to 26 wherein, for 31-*epi*lolitrem B, the half maximal degree of antagonist inhibition (IC_{50}) is found for a composition containing approximately 49 nM of 31-*epi*lolitrem B to inhibit α and β_4 activity.

44. The use as claimed in any of claims 23 to 26 wherein, for lolitrem E, the degree of antagonist inhibition is approximately 100% for a composition containing approximately 100 nM lolitrem E.

45. The use as claimed in any of claims 23 to 26 wherein the antagonist effect of the composition is not able to be reversed by wash out for concentrations of 10 nM or greater of lolitrem B compound.

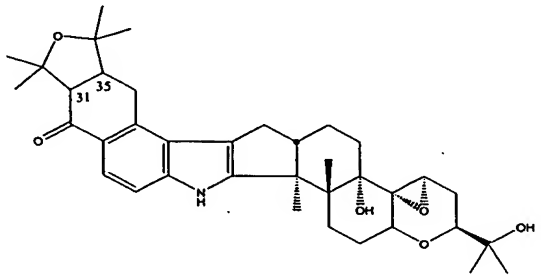
46. A composition that contains a pharmacologically effective amount of at least one BK channel antagonist compound containing the moiety shown in structure (VII):



STRUCTURE (VII)

which includes lolitrem K = 31α, 35β stereochemistry, where R = H or acetate.

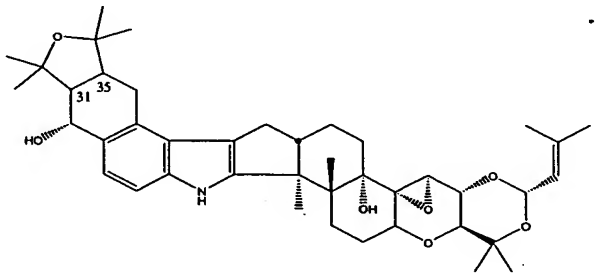
47. A composition that contains a pharmacologically effective amount of at least one BK channel antagonist compound containing the moiety shown in structure (IX):



STRUCTURE (IX)

which includes lolitrem M = 31α, 35β stereochemistry.

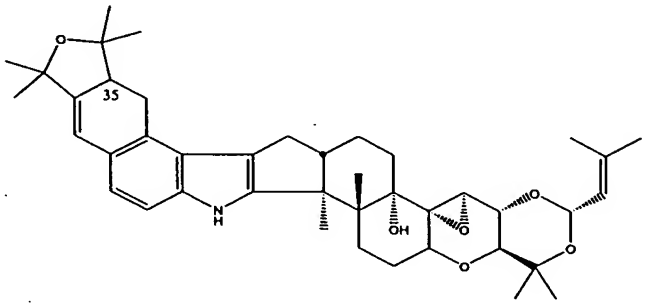
48. A composition that contains a pharmacologically effective amount of at least one BK channel antagonist compound containing the moiety shown in structure (XII):



STRUCTURE (XII)

which includes compounds selected from the group consisting of: 30-desoxylolitrem B-30α-ol = 31α, 35β stereochemistry; 30-desoxy-31-*epi*lolitrem B-30α-ol = 31β, 35β stereochemistry.

49. A composition that contains a pharmacologically effective amount of at least one BK channel antagonist compound wherein the antagonist compound is structure (XIII):



STRUCTURE (XIII)

which includes 30-desoxylolitrem B-30-ene = 35β stereochemistry.